BEST AVAILABLE COPY

(12) UK Patent Application (19) GB (11) 2 349 534 (13) A

(43) Date of A Publication 01.11.2000

(21) Application No 9909522.6

(22) Date of Filing 27.04.1999

(71) Applicant(s)
Jonathan David Hares
Iden House, Bakers Lane,
BRIGHTWELL-CUM-SOTWELL, Oxfordshire,
OX10 0PY, United Kingdom

(72) Inventor(s)
Jonathan David Hares

(74) Agent and/or Address for Service
Jonathan David Hares
Iden House, Bakers Lane,
BRIGHTWELL-CUM-SOTWELL, Oxfordshire,
OX10 0PY, United Kingdom

(51) INT CL[7]
H04N 5/235, G01N 27/64, H04N 3/15

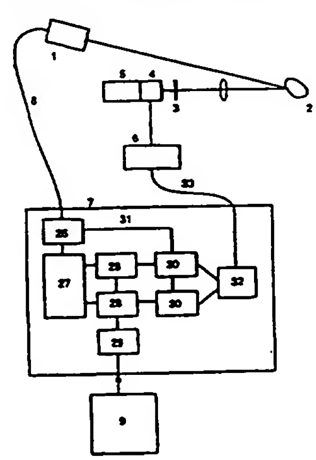
(52) UK CL (Edition R)
H4F FCCY F82A

(56) Documents Cited
GB 2329809 A   GB 2322496 A   GB 2040139 A
EP 0260049 A2   WO 92/13265 A1

(58) Field of Search
UK CL (Edition Q) H4F FCCA FCCY FJA
INT CL[6] G01N 27/64, H04N 3/15 5/235
Online: WPI, EPODOC, JAPIO

(54) Abstract Title
Sinusoidal modulation and detection system

(57) A laser 1 illuminates the object 2 which is imaged via an optical system through the filter 3 onto a proximity focused wafer micro-channel plate (MCP) intensifier 4. A CCD camera 5 integrates images from the output of the intensifier and these are digitized and analyzed. The CCD integration period may be computer controlled and the timing of this period may be synchronized to the illumination source. A programmable trigger sequencer 7 consists of an input trigger and pre-counter circuit 26, a digital counter 27, the maximum count of which may be programmed, a pair of memories 28, addressed by the output of the counter 27, circuitry 29 to allow the data in these memories to be programmed from a controlling computer 9, a pair of delay circuits 30, the delays of which are independently set by the data in the memory, a path 31 from the input circuit 26 to the delay circuits 30 which carries pulses synchronized to the illumination source to the delay circuits and an output circuit 32, which combines the two delayed signals from the delay circuits into an output pulse, the leading and falling edges of which may be timed arbitrarily on each output pulse. The output from the programmable trigger sequencer is fed to a wideband pulse amplifier 6 which amplifies the trigger signal 33 to a level suitable to drive the photocathode of the image intensifier 4. By suitable choice of data programmed into the memories 28 a wide range of effective gain vs time profiles may be obtained, including sinusoidal harmonic profiles of the laser pulse rate up to the maximum defined by the minimum gate width which can be obtained from the gated intensifier. A triggerable illumination source may be used in an alternate embodiment of the invention in which the programmable trigger sequencer is used to program the illumination modulation profile. An RF signal is applied to the detector which yields a fixed modulation pattern with respect to a synchronization signal. This synchronization signal will usually be derived from the applied RF signal itself.



GB 2 349 534

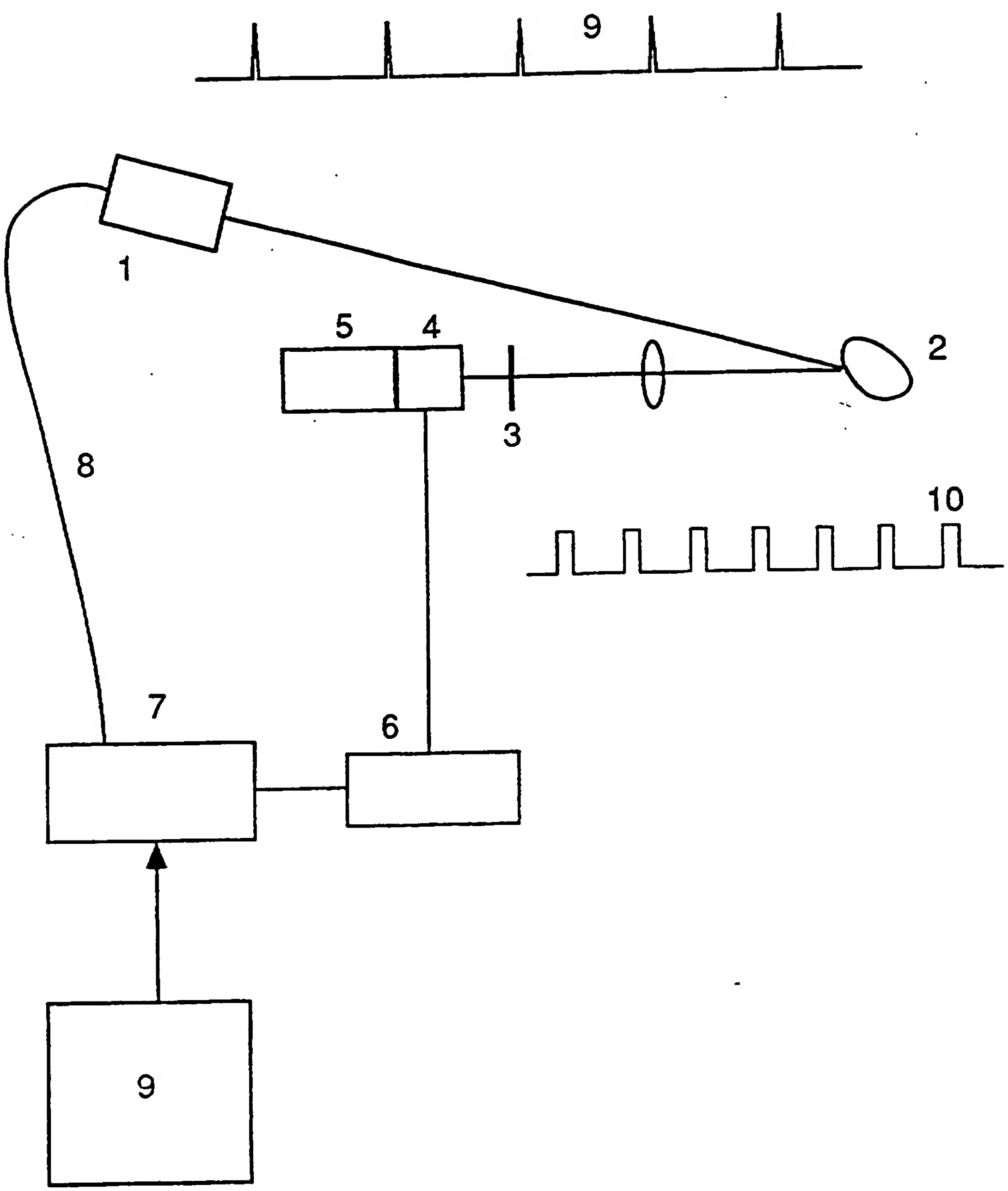


Figure 1

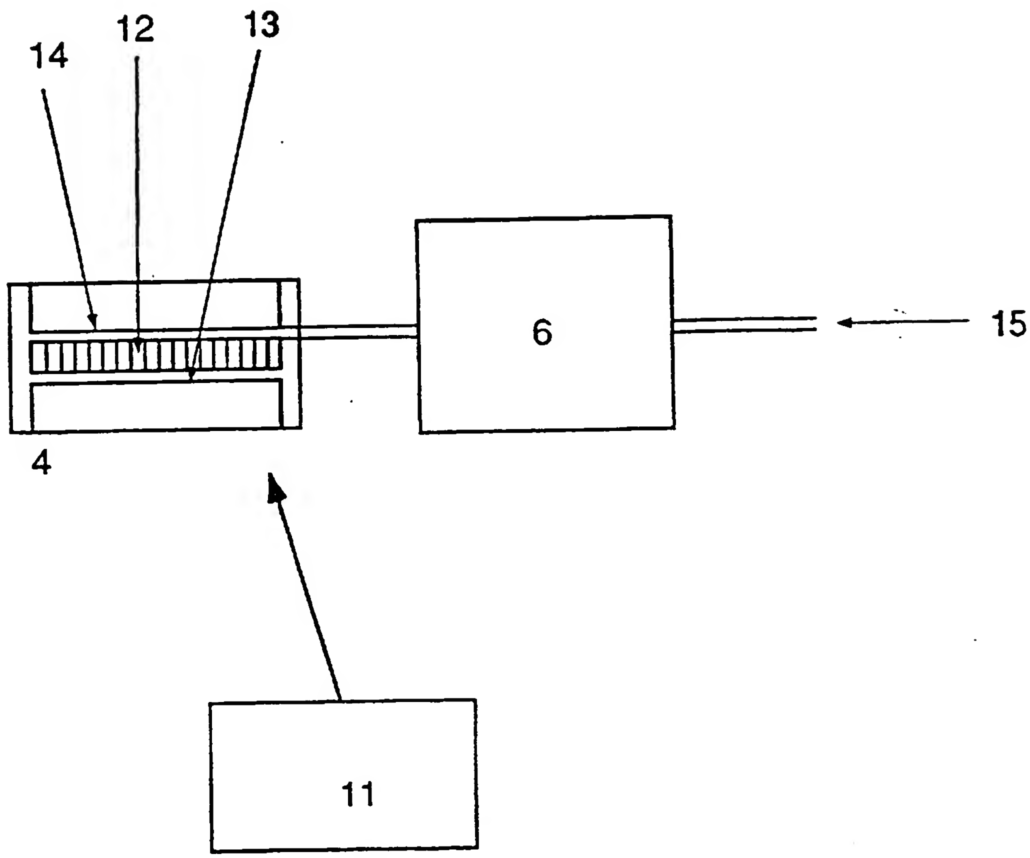


Figure 2

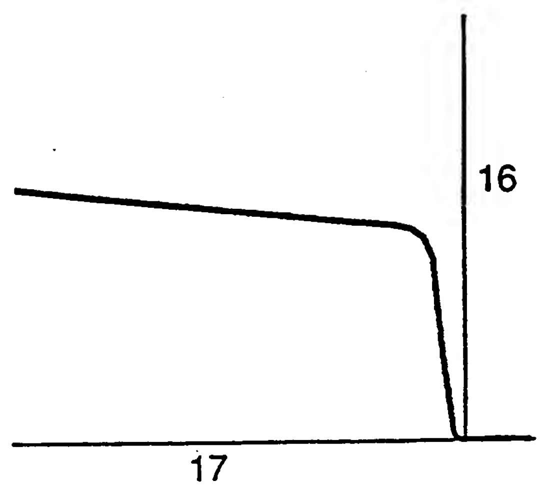


Figure 3

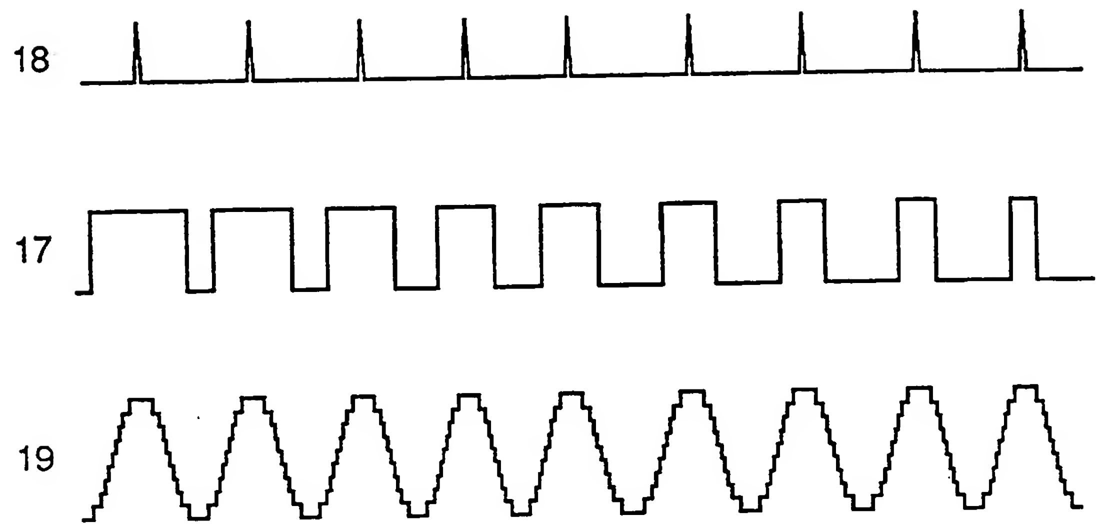


Figure 4

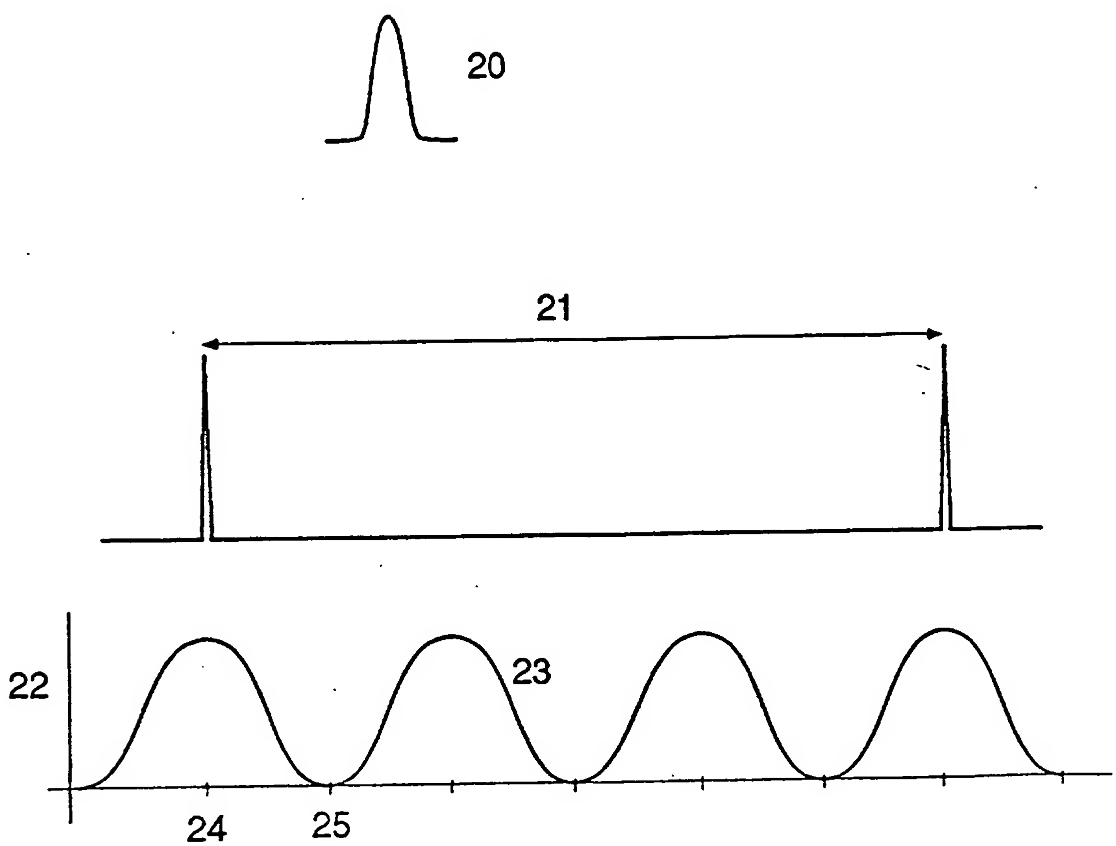


Figure 5

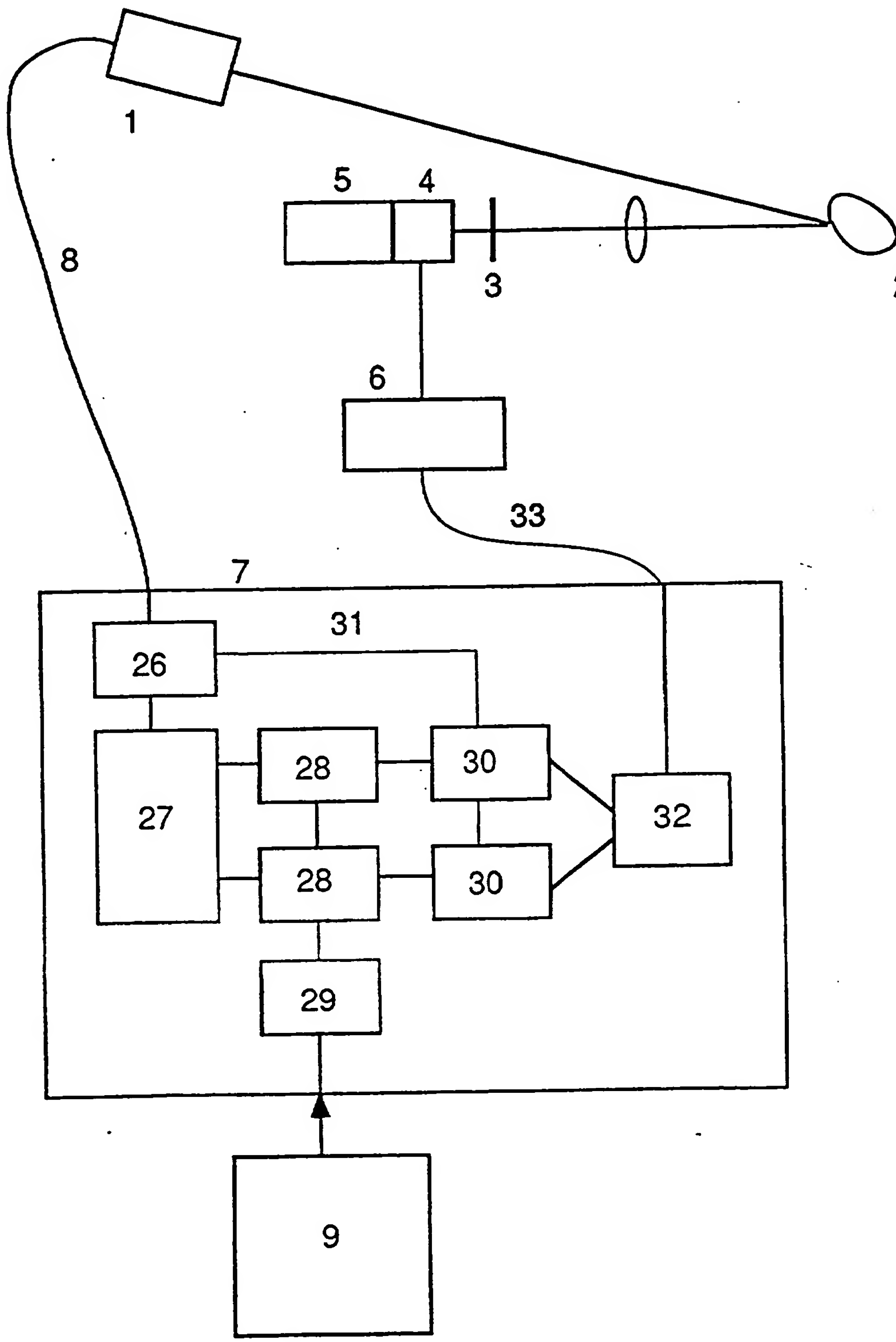


Figure 6

Sinusoidal modulation of illumination and detection apparatus

This invention relates to the linear modulation of illumination sources and imaging detectors at radio frequencies in a manner which may be programmed and which can be chosen to be in a form equivalent to a very pure sinusoid.

A particular application for modulated illumination sources and imaging detectors is in fluorescence life time imaging (FLIM) in which a sample is illuminated with a modulated light source, usually a repetitively pulsed laser, and the fluorescence signal is imaged and analysed with respect to the time dependance of the signal relative to the modulation of the source. Analysis of the time dependance of the fluorescence signal can provide enhanced contrast in the resulting image of the sample. This technique is useful for biological imaging and other applications. The fluorescence lifetimes of interest lead to a requirement for modulation frequencies extending from less than 100kHz to greater than 1GHz.

The analysis of the time dependance of the fluorescence signal may be performed in the frequency domain. Images resulting from non-sinusoidal modulation may be analysed by frequency domain techniques however the analysis is complex. It is desirable to simplify the analysis as much a possible in order that processed images are available quickly. It is helpful to such analysis if the form of the modulation of the source and/or the detector is a pure sinusoid as this allows the application of simple Fourier techniques.

The most common schemes which provide radio frequency (RF) gain modulation use a proximity focussed micro-channel plate (MCP) intensifier although cross-over image converter tubes have been used. The gain of a proximity focussed image intensifier may be adjusted by means of the application of, for example, a sinusoidal voltage to the MCP or the photo-

cathode (PC). In these cases the variation of gain with voltage is non-linear which can lead to harmonic distortion. Alternatively the modulation voltage may be applied to the phosphor however a large voltage is required to give significant modulation depth. It is easier, from an electrical point of view, to drive the PC as the capacitance is lower than the MCP and the required voltage is much smaller than that required to modulate the phosphor, however the extreme non-linearity of the gain as the PC voltage approaches zero restricts the linear modulation depth. Furthermore the spatial resolution of the intensifier becomes low as the PC voltage approaches zero.

In a typical FLIM arrangement a high frequency pulsed laser provides continual illumination of a sample. The sample is imaged via a modulated detector which is driven at various harmonics of the laser frequency. An image is captured at each frequency, each image being integrated over many individual laser pulses.

According to the present invention there is provided a modulated detector, for example an image intensifier with drive electronics allowing it to provide a sequence of exposures, a modulated illumination source, for example a laser, and an electronic means for the generation of a complex and variable trigger signal timed with respect to a reference RF signal. The phase, duration and/or pattern of this trigger signal may be changed with respect to the reference RF signal several times during an image integration period consisting of many illumination and modulation cycles. This trigger signal may be applied to the modulated detector or a modulated illumination source for the purpose of providing a sequence of illumination and detection cycles in which the illumination and/or detection modulation patterns change during an integration period such that the integrated image obtained is the same as if a chosen linear modulation function were applied to the detector.

A particular embodiment of the invention will now be described by way of

example with reference to the accompanying drawings in which:-

Figure 1 shows an imaging system, a pulsed illumination source and a modulated detector;

Figure 2 shows a particular scheme for providing a modulated intensifier;

Figure 3 shows the typical relationship between intensifier gain and PC voltage;

Figure 4 shows a particular sequence of gate pulses together with the associated illumination pulse train and the resulting effective gain modulation;

Figure 5 illustrates the case in which a single optical gate profile can be used to synthesise substantially pure effective sinusoidal modulation;

and

Figure 6 shows a block diagram of an imaging system embodying the invention.

Referring to figure 1, a pulsed light source 1 illuminates an object 2. The object is imaged via a filter 3 which rejects the scattered original illumination but passes the fluorescence light. A modulated detector 4 detects the image formed by the fluorescence light and an electronic camera 5 integrates the image for a defined period which spans several pulses of illumination. The modulated detector is driven by drive electronics 6 which are triggered by a programmable trigger sequencer 7. A synchronisation signal 8, common to or provided by the light source, triggers the programmable trigger sequencer. The programmable trigger sequencer is programmed by a host computer 9.

As described above the integrated image captured by the camera 5 is a sum of images produced by many individual cycles of illumination and detector

modulation. A consequence of this is that in the case that the form of the intensifier modulation function varies during the integration period the captured image is the same as if the modulation function were the average of that which is actually applied, where the averaging is performed by the superposition of several illumination/modulation cycles.

Figure 2 illustrates a particular scheme for providing a modulated detector. A proximity focussed MCP intensifier 4 is connected to a high voltage supply 11 which provides the required DC operating bias voltages for the MCP 12, phosphor 13 and PC 14. The PC and input connection for the MCP are connected to PC drive electronics 6 which in this case is a wideband pulse amplifier. Usually the MCP input will be grounded with the applied negative gating pulse voltage being applied to the PC. The wideband pulse amplifier 6 is driven by trigger input 15.

Figure 3 shows the typical relationship between intensifier gain 16 and PC voltage 17. The gain rises very abruptly as the PC becomes negative with respect to the MCP input. Thereafter the rise is small. This behaviour leads to the 'squaring' of an applied PC gate voltage. A sinusoidally varying PC voltage will lead to rather square gating behaviour. If a bias is applied such that the PC operates in a 'linear' region of gain variation the modulation depth is restricted.

The wideband pulse amplifier is designed to provide an output voltage which is either high or low in response to the trigger input. When combined with the non-linear behaviour of the gain the intensifier can be considered to be either on or off, the transitions between these states being rapid.

Figure 4 shows a particular sequence of intensifier gate pulses 17 together with the associated illumination pulse train 18. Such a gate pulse sequence, which varies in width from pulse to pulse, can be produced by the application of a suitable trigger signal to the wideband pulse amplifier. Such a trigger signal

will be produced by the programmable trigger sequencer 7. The shutter of the camera 5, be it either mechanical or electronic, will be open for the entire sequence and so the camera integrates the image over the complete sequence. Assuming that the signal from the object of interest is the same after each cycle of illumination, the resulting image will be the same as if the detector were modulated with the average modulation function 19. The example illustrated shows a poor approximation to a sinusoidal function however by increasing the integration time, and hence increasing the number of modulation cycles which are superimposed, arbitrarily pure sinusoids may be synthesised. By suitable choice of complex trigger signals a wide range of effective gain modulation profiles may be produced, including those which consist of high purity sinusoids.

The highest frequency linear modulation which may be synthesised is determined by the half width of the minimum intensifier gate which can be produced and the half width of the narrowest illumination pulse. Lasers are able to produce sub picosecond pulses of light and MCP intensifiers have produced optical gates of less than 0.5 nanoseconds at high repetition rates which allows the synthesis of substantially pure sinusoidal modulation profiles with large modulation depths at frequencies in excess of 1GHz.

A particular means for the realisation of a programmable trigger sequencer uses straightforward electronic circuits including a digital memory as a look-up table for the trigger sequence parameters, a counter to allow the sequential access of the memory, a trigger circuit to detect the synchronisation signal from the laser, camera or other master RF source together with a computer interface to allow the sequence to be programmed.

In the embodiment described above the modulated illumination source produces a constant modulation pattern, such as a train of narrow impulses, and the variation during the integration period has been provided by varying the

detector timing and gate profile. There is in fact interchangeability between the illumination modulation pattern and the intensifier modulation pattern. This occurs as the average signal which appears at the detector depends on the convolution of the illumination modulation pattern and the detector modulation pattern. This allows that the patterns may be swapped (and phased appropriately) to give the same recorded signal. This reversal is also illustrated by Figure 4 but where the labels for illumination and detection patterns are swapped i.e. 18 indicates a sequence of narrow intensifier gate pulses and 17 indicates a programmed illumination pulse sequence. In this case the illumination source must be such that it can be triggered. This numerical equivalence allows for the invention to be applied equally well by the use of the programmable trigger sequencer to drive the illumination source where the detector is operated at a substantially constant frequency and with a fixed modulation pattern.

The principal of the invention may be summarised as follows. A modulated detector is to be used with a modulated illumination source. The detector and illumination source each provide certain modulation characteristics however these characteristics are such that linear control of the gain or illumination over a wide range at high frequencies is impractical. The detector is used in an application where many cycles of illumination and detection are integrated together and in which particular illumination and/or detection modulation functions are required, typically those which are sinusoidal functions of time. The form of a complex trigger signal which is applied to the detector and/or the illumination source is chosen such that the superposition of the contribution to the image from several illumination/detection cycles sums to the signal which would have been obtained if the required modulation had been available from the detector and/or illumination source. The summation is performed over an integration period consisting of several illumination and detection cycles and the modulation function changes during that period. The form of the modulation may be programmable such that different effective gain vs time

profiles can be obtained during each integration period under computer control. It is a special case in which the effective gain varies sinusoidally with time.

It should be noted that the persistence of phosphors used in image intensifiers is usually much greater than typical periods between illumination pulses in a fluorescence imaging system so the integration of several illumination cycles is more or less automatic in such a system.

Although the description above refers to an intensifier which is either on or off, the invention applies equally well to a gated intensifier system in which the actual gate profile has a temporal shape other than a rectangular one. This will be the situation when, for example, the intensifier is operated with its minimum gate width. The only mathematical requirement on the form or forms of the effective intensifier gate profile which can be achieved is that the required effective gain vs time behaviour can be constructed by the superposition of many intensifier gate profiles. It is this condition which leads to the earlier statement that the highest frequency which may be synthesised is set by the half width of the minimum intensifier gate which can be produced.

Figure 5 illustrates the case in which a gated intensifier system can provide only a single optical gate profile 20 with a roughly gaussian shape. An effective sinusoidal modulation can be obtained by suitable variation of the timing of the optical gate with respect to the pulsed illumination source (or vice versa). The pulsing of the illumination source has a period 21 between pulses. The effective gain 22 of the detector is to follow the sinusoidal profile 23, being, in the case illustrated, the third harmonic of the illumination source frequency. On each successive pulse the of illumination source the intensifier gate pulse 20 will be positioned somewhere in the period 21 between successive illumination pulses. The detected image will be the integrated sum of many such gate cycles. The sequence of the timing of the intensifier gate 20 during the integration period is

arranged such that the gate occurs frequently at times where high gain is required 24 and infrequently where little gain is required 25. There is of course a mathematical prescription for this timing which may be obtained simply from the required effective gain profile. The precision with which the effective gain profile is constructed determines how many illumination cycles the integration period spans.

In general it is possible to synthesise an effective gain vs time behaviour which has close to 100% modulation depth. In order to obtain such great modulation depth by direct means the intensifier must be driven into the non-linear region and harmonic distortion will result.

The principal aim of this invention is to obtain images with particular effective temporal modulation functions from a detector and illumination source which do not readily provide linear modulation over a wide range. Furthermore such images may be obtained without the need for mathematical processing of the image after capture. Typically the effective temporal modulation function will be sinusoidal. Furthermore the invention allows the frequency and form of the effective modulation to be chosen at random and under computer control.

The use of a specially designed programmable trigger sequencer is a particular means by which the variation of the intensifier gate or illumination pattern may be achieved between illumination pulses during the integration period. Other means include the use of analogue delay circuitry to vary the sequence trigger signal, varying the DC bias voltage on the intensifier photocathode to change the intensifier gating profile and varying the timing of the pulsed illumination source.

This description refers to gated wafer MCP intensifiers however the invention may be applied equally well to other electron-optical shutter tubes or any modulated imaging detector.

Figure 6 illustrates an application of a preferred embodiment of the invention. A laser 1 illuminates the object 2 which is imaged via an optical system through the filter 3 onto a proximity focussed wafer MCP intensifier 4. A CCD camera 5 integrates images from the output of the intensifier and these are digitised and analysed. The CCD integration period may be computer controlled and the timing of this period may be synchronised to the illumination source. A programmable trigger sequencer 7 consists of an input trigger and pre-counter circuit 26, a digital counter 27, the maximum count of which may be programmed, a pair of memories 28, addressed by the output of the counter 27, circuitry 29 to allow the data in these memories to be programmed from a controlling computer 9, a pair of delay circuits 30, the delays of which are independently set by the data in the memory, a path 31 from the input circuit 26 to the delay circuits 30 which carries pulses synchronised to the illumination source to the delay circuits and an output circuit 32, which combines the two delayed signals from the delay circuits into an output pulse, the leading and falling edges of which may be timed arbitrarily on each output pulse. The output from the programmable trigger sequencer is fed to a wideband pulse amplifier 6 which amplifies the trigger signal 33 to a level suitable to drive the photocathode of the image intensifier 4. By suitable choice of data programmed into the memories 28 a wide range of effective gain vs time profiles may be obtained, including sinusoidal harmonic profiles of the laser pulse rate up to the maximum defined by the minimum gate width which can be obtained from the gated intensifier.

A triggerable illumination source may be used in another preferred embodiment of the invention in which the programmable trigger sequencer is used to programme the illumination modulation profile. An RF signal is applied to the detector which yields a fixed modulation pattern with respect to a synchronisation signal. This synchronisation signal will usually be derived from the applied RF signal itself. A programmable trigger sequencer is used to

generate a sequence trigger signal from the synchronisation signal. The sequence trigger signal is applied to a triggerable modulated light source, such as a light emitting diode or laser diode, which is used to illuminate the object. Choice of a modulation pattern, exactly as described above with respect to figure 4, allows effective substantially pure sinusoidal illumination and/or gain modulation to be synthesised at frequencies up to those set by the half width of the shortest duration illumination pulse and the shortest duration intensifier gate.

In the case that the voltage appearing on the photocathode is rectangular the spatial resolution may be enhanced with respect to conventional sinusoidal voltage drive as, during the time for which the intensifier is active, the cathode is nearly always at maximum voltage.

Claims

1 A modulated optical detection system in which images of an object repeatedly illuminated with a modulated illumination source may be acquired such that the average effective gain vs time behaviour of the detector may be arbitrarily programmed over a wide range of functions, including sinusoids, by means of varying the temporal profile and/or relative timing of the optical gate of the detector and/or a trigger signal provided for the triggering of an illumination source during an acquisition period in which many illumination and detection cycles are integrated into an output image.

2 A modulated optical detection system as in claim 1 in which is provided a means to generate a complex trigger sequence signal, the initiation of which is synchronous with a modulated illumination source and/or a modulated detector.

3 A modulated optical detection system as in claim 2 in which a complex trigger sequence signal is used to control a modulated detector and/or a modulated illumination source.

4 A modulated optical detection system as in claim 3 in which the complex trigger sequence signal may be programmed and may be changed between cycles of the modulated illumination source and/or the modulated detector.

5 A modulated optical detection system as in claim 4 in which a modulated illumination source provides a sequence of short impulses of illumination.

6 A modulated detection and illumination system as in claim 4 in which the detector is an electron-optical image converter tube

7 A modulated optical detection system as in claim 6 in which the modulation is provided by the application of a modulation voltage to an electrode in an electron-optical image converter tube.

8 A modulated optical detection system as in claim 6 in which the modulated detector is a proximity focussed microchannel plate intensifier tube driven by an electronic amplifier or pulse amplifier connected to the photocathode.

9 A modulated optical detection system as in claim 8 in which a programmable electronic means for triggering the pulse amplifier is provided such that the modulation signal may be changed between cycles of the modulated illumination source.

10 A modulated optical detection system as in claim 4 in which a programmable electronic means for triggering a modulated illumination source is provided such that the modulation illumination signal may be changed between cycles of the modulated detector.

11 A modulated optical detection system as in claim 11 in which a programmable complex trigger sequence signal is used to control a triggerable illumination source and in which a modulated detector comprising an image intensifier is modulated at a substantially constant frequency throughout an image integration period.

12 A modulated optical detection system for use with a modulated

illumination source in which the average effective illumination and/or modulation behaviour may be programmed over a range of functions of time, including sinusoids, by the superposition of the contribution to the image from several different illumination/detection cycles with different modulation patterns, substantially as hereinbefore described.

The Patent Office

INVESTOR IN PEOPLE

14.

Application No: GB 9909522.6
Claims searched: All

Examiner: Joe McCann
Date of search: 1 September 1999

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): H4F(FCCA, FCCY, FJA)

Int Cl (Ed.6): G01N(21/64); H04N(3/15,5/235)

Other: Online: WPI, EPODOC, JAPIO

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	GB 2329809A	(GEC-MARCONI) - see abstract	1
X	GB 2322496A	(MATSUSHITA) - see abstract	1
X	GB 2040139A	(SPERRY CORP) - see abstract	1
X	EP 0260049A2	(WEB PRINTING CONTROLS CO, INC) - see abstract	1
X	WO 92/13265A1	(LAKOWICZ. J) - see abstract	1

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.